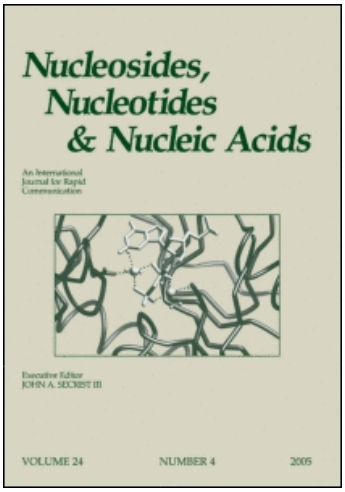

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Synthesis of Analogues of 5'-Mono-,5'-Di-, and 5'-Triphosphate-AZT for the Development of Specific Enzyme Immunoassay for Monitoring of Intracellular Levels of AZT-MP, AZT-DP, and AZT-TP

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SYNTHESIS OF ANALOGUES OF 5'-MONO-, 5'-DI-, AND 5'-TRIPHOSPHATE-AZT FOR THE DEVELOPMENT OF SPECIFIC ENZYME IMMUNOASSAY FOR MONITORING OF INTRACELLULAR LEVELS OF AZT-MP, AZT-DP, AND AZT-TP.

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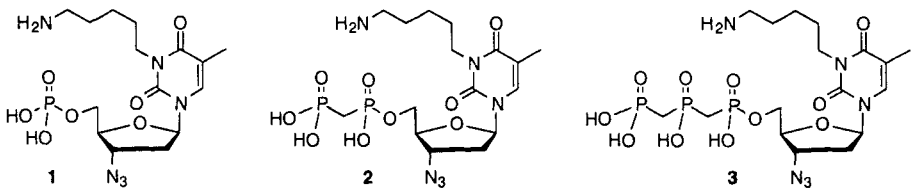
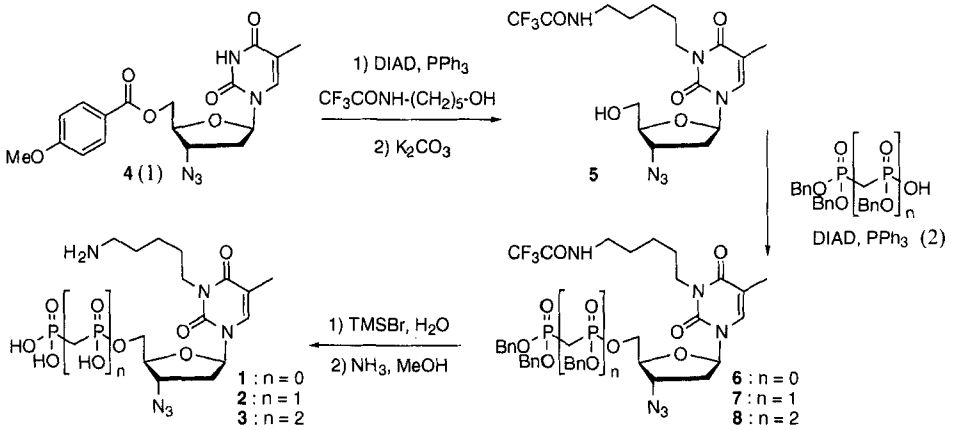
ABSTRACT : The synthesis of analogue compounds of polyphosphorylated AZT is described. The compounds are designed to raise specific antibodies against AZT-MP, -DP and -TP in rabbits.

AZT and 2',3'-dideoxynucleosides (ddNs) were the first drugs to demonstrate significant antiviral activity in the treatment and prevention of AIDS. Nevertheless they do not have any intrinsic activity against HIV and must be metabolized successively into their mono-, di-, and triphosphate derivatives by intracellular kinases and nucleotidases to inhibit competitively the reverse transcriptase of the virus. The rate of formation of dideoxynucleotides (ddNPs) is subject to large interindividual variations and many studies indicate an absence of correlation between ddN circulating levels and the efficacy of treatments. The monitoring of ddNPs is also relevant in terms of toxicity, which is essentially related to the inhibition of cellular polymerases by ddNPs. The measurement of intracellular levels of ddNPs should allow optimization of ddNs-based treatments both in terms of efficacy and toxicity.



- Figure 1 -

In order to develop specific enzyme immunoassays for measuring intracellular concentrations of AZT-MP, AZT-DP, and AZT-TP, derivatives of these compounds were designed (Fig. 1). Since nucleotides are haptenic molecules, they have to be covalently coupled to a carrier protein in order to induce the production of specific antibodies in animal host. The way the hapten will be linked to the antigen (here on the base) is critical because it influences the specificity of the antibodies raised. The synthesis of haptens for di- and triphosphate derivatives is more complicated due to the instability of the polyphosphate chain which is subject to either chemical or enzymatic hydrolysis. In order to overcome that particular difficulty, stable phosphonate analogues of AZT-DP and AZT-TP were prepared (Fig. 2).



- Figure 2 -

The immunogens were prepared by coupling **1-3** with keyhole limpet hemocyanin (KLH) using glutaraldehyde. Acetylcholinesterase (AChE) conjugates (enzymatic tracers) were obtained by reacting compounds **1-3** with SMCC and maleimido-AChE (3). Preliminary results obtained in immunizations indicate that the antibodies raised are very sensitive and specific for AZT-MP (4), AZT-DP, and AZT-TP (respectively).

References

1- Czerneki, S.; Valéry, J.M. *Synthesis*, **1991**, *36*, 239-240.
2- a) Saady, M.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.*, **1995**, *60*, 2946-2947. b) Saady, M.; Lebeau, L.; Mioskowski, C. *Helv. Chem. Acta*, **1995**, *78*, 670-678.
3- Grassi, J.; Frobert, Y.; Pradelles, P.; Chercuite, F.; Gruaz, D.; Dayer, J.M.; Poubelle, P.E. *J. Immunol. Methods*, **1989**, *123*, 193-210.
4- Goujon, L.; Brossette, T.; Dereudre-Bosquet, N.; Creminon, C.; Clayette, P.; Dormont, D.; Mioskowski, C.; Lebeau, L.; Grassi, J. *J. Immunol. Methods* (in press).